Evaluation of elemental, microbial and biochemical status of raw and pasteurized cow's milk

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Abstract

Milk is a good source of nutrients for the children as well as for adult. In the present study, the concentration of lead, cadmium, chromium, copper, cobalt, nickel, arsenic, mercury, manganese, iron, zinc, calcium and magnesium were measured in both raw and pasteurized cow's milk by Atomic Absorption Spectroscopy (AAS). Results showed that pasteurized milk was more nutritious with significantly higher (P<0.05) concentration of Ca and Mg and was safer with significant lower concentration of Pb, Cd, and Ni compared to raw milk. In raw milk, Pb had the highest elemental concentration followed by others in the order Ni > Co > Cr > Cd > Cu. Values of As and Hg were below the AAS detection limit for both raw and pasteurized milk samples. The Daily Intake of Metal and Health Risk Index of the elements calculated from the obtained metal concentrations showed no potential to cause health risk to the consumer. Besides elemental analysis, chemical composition and microbial load in cow's milk were evaluated. The average Total Soluble Solids values of raw and pasteurized milk were 10.26 and 9.87% respectively. Acidity in both raw and pasteurized milk were in acceptable limit (<0.14). Microbiological load in pasteurized milk was less than standard limit (not exceeding 20,000 CFU/ml). Therefore, pasteurized milk from prescribed farm is good quality and safe for human consumption.

Introduction

Milk and milk products are rich and convenient sources of natural nutrients for human being which contains more than twenty different trace elements. Many of them are essential trace elements and play important roles in biological systems in human body (Schuhmacher *et al.*, 1991; Pennington *et al.*, 1995). Some of them form an integral part of several enzymes. Although they are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element to element and from species to species. The contamination of milk with undesirable substances via animal feeds, toxic metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health due to their toxic effects on human and wildlife.

Recently, metal residues in milk are of particular concern because milk is largely consumed by infants and children. Significant amounts of toxic metals can be transferred from contaminated soil to plants and grass, causing accumulation of toxic metals in grazing ruminants, particularly in cow milk. To determine the concentrations of metals in milk could be an important indicator of milk hygiene status as well as an indicator of the degree of pollution in environment where the cattle raise. Metal concentrations in the environment grow with the increase of urban, agricultural, and industrial emissions. The presence of heavy metals in food as arsenic, cadmium, lead and mercury even in low concentrations, leads to metabolic disorders and it causes many health problems such as weakness, heart failure, cancer and also affects the kidneys (Licata *et al.*, 2004).

Milk is also contaminated by the microbial agents during processing, transportation, storage and preparation of milk products for consumption. Microbes have access to milk to reproduce at a rapid rate. Moreover, adulteration of milk with water, which is very common in Bangladesh, not only causes dilution of milk reducing the milk solids, but also involves the risk of introducing germs into the milk, further decreasing its quality (Chanda *et al.*, 2012).

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Therefore, to maintain safety of milk it is important that milk should be obtained from healthy animals as well as collected and stored in safe conditions free from environmental contamination. Pasteurization is the process of heat processing a liquid or to kill pathogenic bacteria to make the food safe to eat. Pasteurization improves the quality of milk and milk products with a longer shelf life by destroying undesirable enzymes and eliminates bacteria. Still there is a chance to contaminate the milk after pasteurization, usually due to poor processing and unsanitary handling of the milk.

In the present study, the farm, Central Cattle Breading and Dairy Farm has been selected because it is located at an industrial zone (Savar, Dhaka) with garments, textile mills, tannery and leather industries, metal products, electronic goods, paper products, chemicals and fertilizers and miscellaneous products which provide a wide range of potentiality for economic development (Khan *et al*., 2011). Savar accommodates the highest number of conventional brick fields emitting black smoke into the air while the Export Processing Zone and other localized industries still discharge wastes into the nearby water bodies and low lands. These are considerable sources of metal contamination to the soil and grass of the Savar area. Another reason for selecting the farm is that most of the inhabitants of this area are low paid industrial or garments workers. They always try to buy cheaper food. That is why they buy milk from the studied dairy farm which is the only government funded dairy farm selling milk at a considerable cheaper rate compared to other private dairy farms. In addition, this dairy farm has been playing a pioneer role for qualitative improvement of cattle through artificial insemination programme in Bangladesh. An average daily milk sale of this dairy farm is 2×10^3 liters/day and market share is 1.08% (Saha and Haque, 2001). However, the concentration of toxic metal in cow's milk should be monitored to ensure the consumers' health of the selected area.

Therefore, the objectives of the present study were to monitor the levels of various essential and toxic trace elements in raw and pasteurized cow's milk from selected farm. Daily Intake of Metals, as well as potential health risk was also investigated. Moreover, the chemical composition and the presence of microbial population in both raw and pasteurized cow's milk were studied to evaluate the nutritional status of the tested milk samples.

Materials and Methods

Milk sampling site and collection

The cow's raw and pasteurized milk was collected from Central Cattle Breading and Dairy Farm, Savar, Dhaka, located at 23.46°-23.58°N, 90.12°-90.20°E in the central part of Bangladesh (Figure 1). This farm has been playing a pioneer role in qualitative improvement of cattle through artificial insemination program in Bangladesh. The farm size was 526.09 hector. Herd size was 1200. The cows of the farm were fed roughages-dry straw and silage and drank ground water. Milking was done manually twice a day at 7.00 am and 5.00 pm.

All samples of raw cow's milk (1 liter) were collected from the bulk milk tanks of the farms into cleaned and sterile washed plastic bottles within 20 min of milking at ambient temperatures during morning milking of a test day and immediately placed in a cooler box. Sampling procedure was repeated three times in same condition at three different test days. Pasteurized milk was sampled after pasteurization at 63°C temperature around 30 min. The collected milk samples were analyzed for biochemical and microbiological assay immediately after arrival at the laboratory. The milk samples were refrigerated at -20ºC and were kept until the trace elemental analysis procedure. Three replicate determinations were carried out on each sample.

Titratable acidity (TA)

Normally *Lactobacillus* is grown in milk that produces more or less lactic acid. In the acidity test the acid is neutralized with 0.1N Sodium hydroxide using phenolphthalein as an indicator. The percentage of lactic acid was calculated from the required amount of alkali (Aggawala and Sharma, 1961).

Total soluble solids (TSS) and pH

TSS was measured by Abbe refractometer (Hanna, Romania) and pH of the sample was measured by digital pH meter (Jenway, UK).

Nutritional quality analysis

The percentage of protein, fat, Solids non-fat (SNF) and lactose of milk were determined by using the Lactostar auto milk analyzer (Funke-Gerber, Berlin, Germany). Triplicate determinations were carried out on each biochemical analysis.

Microbial analysis of milk

The collected milk samples were analyzed for microbial status immediately after arrival at the laboratory. Total Bacterial Count (TBC) was enumerated by the Standard Plate Count (SPC) method following the method described by Sharp and Lyles (1969). Nutrient agar (DifcoTM, USA, pH 7.0-7.4) was used to determine TBC as well as for isolation purposes. Appropriate dilution of raw milk sample was prepared using 0.9% Sodium chloride solution and pasteurized milk sample was directly

inoculated on these media without any dilution. Thereafter, 100 µl of each sample was inoculated in respective culture media using sterile pipette and spreaded on medium using a sterile glass spreader for each sample. Plates were then kept in an incubator at 37ºC for 24-48 hours. Following incubation, plates exhibiting colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TBC. Microorganisms associated with milk sample were expressed as the number of organism of colony forming units per millilitre (CFU/ml). Total Viable Coliform Count (TCC) was done in the same way using Mac Conkey agar (Acumedia, USA) medium at 37ºC. De Man, Rogosa and Sharpe media (Sigma-Aldrich, Switzerland) was used to enumerate total *Lactobacillus* count. Viable cell counts (CFU/ml) were the average of at least three independent experiments.

Chemicals and equipment used

Individual standard solution (Spectropure, USA) of target element for AAS analysis supplied by Varian Inc, USA with highest purity level (99.98%) was used and the acid matrix matched to the samples. Supra pure HNO_3 from E. Merck, Germany and all other Chemicals were extra pure or supra pure from E. Merck, Germany were used for the analysis. Flame (model no: Varian AA240 FS), Hydride-Generation (model: Varian AA240), Cold-Vapour (model: Analytik Jena, NovAA 350) atomic absorption spectrometers were used as major analytical techniques for trace element determination.

Milk samples digestion for metal analysis

Approximately 9 litre milk samples were placed in acid cleaned polyethylene bottles and were shipped to laboratory with rush delivery. To determine the concentration of metals, 1 gm milk was taken in a XP vessel where 6 mL of concentrated HNO_3 was poured. Samples were then digested in a microwave accelerator reaction system (Model No: MARS 5) following USEPA procedure 3051A. The heating programs of MARS are as follows: Ramp time: 15min, pressure: 800 psi, Temp: 200°C Hold: 15min, power 800 w (for 6 vessels). After digestion, the solution was made up to 10mL using ultra-pure water (>18 MΩ•cm). Finally, the samples were analyzed with AAS for metal estimation.

Daily intake of metals (DIM)

Daily intake of metal was calculated by the following equation:

Daily intake of metal (DIM) = (C metal × D food intake) / B average weight

Where Cmetal, Dfood intake, and Baverage weight represent the heavy metal concentrations in milk (mg/L), daily intake of milk (L) and average body weight, respectively. The average milk intake of population of Bangladesh is 30.56 ml/person/day (Nahar *et al.*, 2013), the average body weight (Baverage weight) was taken as 70 kg for adults according to WHO (1993).

Health risk index (HRI)

The Health Risk Index was calculated as the ratio of Daily Intake of Metals through concerned food and oral Reference Dose (RfD) (Cui *et al.*, 2004). RfD is the safe intake limit of a metal per day having no hazardous effect during life time (Integrated Risk Information System of the U.S. Environmental Protection Agency-USEPA IRIS, 2006).

Oral Reference Doses used for calculating HRI for Cr, Ni, Cu, Pb, Cd, Mn and Zn were followed by USEPA IRIS (2006), for Co followed by Food and Nutritional Board (2004) and for Fe followed by Friberg *et al.*,(1984). The health risk index for Cr, Co, Cu, Mn, Fe, Zn, Ni, Pb was calculated by the following equation (Cui *et al.*, 2004):

HRI= DIM / RfD

Hazard index (HI)

To evaluate the overall health risk through more than one heavy metal of an individual food item, the Hazard Index (HI) was developed (USEPA, 1996). The Hazard Index is the sum of the hazard quotients (Health Risk Index) as described in the following equation:

HI = ∑ HQ = HQCr+ HQCo+ HQCd+ HQPb+HQNi+HQMn+HQZn+HQCu+HQFe

Like HRI, HI < 1 indicates that the predicted exposure is unlikely to pose potential health risks. However, a hazard index >1 does not necessarily indicate that a potential adverse health effects will result, but only indicates a high probability of posing health risks.

Statistical analysis

All data were analyzed by the Student's t-test and expressed as an average and also mean standard deviation. Differences were considered significant at the level of P<0.05.

Table1. Biochemical analysis of Raw and Pasteurized milk

Biochemical composition	Raw	Pasteurized
Acidity (%)	0.14±0.025[a]	0.11±0.063[a]
pH	6.91±0.003[a]	6.84±0.035[a]
TSS (%)	10.26±0.225[a]	9.86±0.225[a]
Protein (%)	3.85 ± 0.02[a]	3.60 ± 0.05[b]
Fat (%)	4.39 ± 0.03[a]	4.13 ± 0.0[b]
Lactose (%)	5.57 ± 0.03[a]	5.26 ± .01[b]
Solid non-fat (%)	10.25 ± 0.06[a]	9.68 ± 0.01[b]

Values are the mean of three replicates; SD =Standard Deviation

Results and Discussion

Biochemical analysis of whole milk

Physico-chemical and nutritional properties were measured to assess milk quality. Results of titrable acidity, pH, Total Soluble Solid (TSS), Solids non-fat (SNF), total protein, total fat and lactose contents of the raw and pasteurized milk are shown in Table 1. The pH indicates precisely about the freshness state of the milk. Fresh milk pH should be neutral or slightly acidic. According to the present study, pH value of raw and pasteurized milk was between the ranges of 6.8-6.9. Statistical analysis showed that there was no significant difference (<0.05) between pH value of raw and pasteurized milk. Some researchers also found the identical findings in camel, cow and buffalo milk (Ibtisam and Marowa, 2009; Agged *et al.*, 2010; Batool *et al*., 2012). The analysis using one sample t-test revealed no significant differences between raw (10.27%) and pasteurized milk (9.87%) for Total Soluble Solid (TSS). The present TSS values were lower than Bangladesh Standard of Dairy Product (BDS) standard by BSTI (1985). The present values are also lower than the average total solids (18.10±1.40 w/w) content of raw cow milk from Tamil Nadu, India (Lingathurai *et al*., 2009). Type of breeds, feeding systems, milking frequency, milking method, seasonal changes and lactation period may be exert an effect on the TSS values (Suman *et al.*, 1998).

Measurement of acidity in milk is an important factor in judging milk freshness and it also affects taste as well. Less than 0.14% acidity ensures the high quality of milk (Popescu and Angel, 2009). The average acidity for raw milk was 0.14% and for pasteurized milk was 0.11% where Bangladesh standard (BSTI, 2002) allows a maximum acidity of 0.15% for the pasteurized milk. The SNF contents in raw and pasteurized milk were 10.25 and 9.68% respectively which were higher than Food and Drug Administration (FDA) standard (8.25%) for SNF

Table 2. Quantitative assessment of microorganisms (Count of viable microorganisms) in raw and pasteurized milk (CFU/ml)

Raw Milk			Pasteurized Milk		
TBC	TCC	TLC	TBC	TCC	TLC
2.37×10^5	2.1×10^4	2.9×10^4	3.5×10^2	Nil	1.4×10^1
2.45×10^5	3.0×10^4	3.9×10^4	3.88×10^2	Nil	1.5×10^1
2.31×10^5	2.4×10^4	3.5×10^4	3.97×10^2	Nil	1.7×10^1

TBC- total bacterial count; TCC- total coliform count; TLC- total *Lactobacillus* count

(Graf, 1976). The statistical analysis showed that SNF content between raw and pasteurized milk was significantly different. Results (Table 1) illustrated that fat, protein and lactose content were significantly higher in raw milk than the pasteurized milk. The same trend recorded for raw milk was observed with milk pasteurization in cow's milk (Enb *et al.*, 2009).

Microbiological evaluation

In this study, microbial analysis was also carried out to investigate the level of microbiological quality of raw and pasteurized cow's milk samples (Table 2). The ranges of Total Bacterial Count (TBC) was varied from 2.31×10^5 to 2.45×10^5 CFU/ml in raw milk. Almost similar results was observed previously in raw milk by Iknomov *et al*. (1956) who reported that the total bacterial counts in raw milk depend on milking techniques and cleanliness. Aaku *et al.* (2004) reported the presence of 5.5×10^6 CFU/ml of the total number of micro-organisms in raw milk which is higher than our result the present study. Lee *et al.* (1983) found that the bacterial count ranged from 4×10^6 to 2.7×10^7 CFU/ml in raw milk. The Total Coliform Count (TCC) and Total *Lactobacillus* Count (TLC) of raw milk samples were varied from 2.1×10^4 to 3.0×10^4 CFU/ml to 2.9×10^4 to 3.9×10^4 CFU/ml. The incidence of coliforms in raw milk might due to their association with contamination of fecal origin and their growth in raw milk at ambient temperature. Coorevits *et al.*, (2008) demonstrated that different sources such as air, milking equipment, feed, soil and grass are involved for the presence of a higher number of micro-organisms leading to microbial contamination in raw milk. Recent studies suggested that the infection occurred subsequently from the skin of animals, milker's hands, cow shed and milking utensils as well as the quality of farm water and other hygienic conditions might be responsible for microbial quality of raw milk. In pasteurized milk, TBC and TLC ranged from 3.5×10^2 to 3.9×10^2 CFU/ml and 1.4×10^1 to 1.7×10^1 CFU/ml respectively. No TCC count in any of the

Table 3. Concentration of trace elements in raw and pasteurized milk

Sample	Pb (mg/L)	Cd (mg/L)	Cr (mg/L)	Cu (mg/L)	Co (mg/L)	Ni (mg/L)	As (mg/L)	Hg (µg/L)	Mn (mg/L)	Fe (mg/L)	Zn (mg/L)	Ca (mg/L)	Mg (mg/L)
Raw milk	3.875 ± 0.066[a]	0.374 ±0.007[a]	1.204 ±0.073[a]	0.17 ±0.017[a]	1.02 ± 0.056[a]	1.75 ± 0.11[a]	< 0.003	< 0.3	0.2 ±0.03[a]	10.02 ±0.43[a]	2.72 ± 0.035[a]	617.41 ±4.82[a]	45.23 ±0.48[a]
Pasteurized milk	3.37 ± 0.064[b]	0.309 ±0.007[b]	1.24 ± 0.069[a]	0.24 ± 0.018[b]	1.48 ± 0.025[b]	1.51 ± 0.09[a]	< 0.003	< 0.3	0.21 ±0.015[a]	10.37 ±0.06[a]	3.77 ± 0.02[b]	756.51 ±7.55[b]	51.30 ±0.23[b]

Values are the mean of three replicates; SD =Standard Deviation

pasteurized milk samples was found. The results obtained during this work showed that the average number of microbes in pasteurized milk samples are less than that recommended value by Bangladesh Standards and Testing Institution and U.S. Public Health Service (not exceeding 20,000 CFU/ml) (BSTI, 2002; Jay, 2003). The reason for bacterial count in the pasteurized milk of this study might be due to poor bacteriological quality of milk and inadequate plant cleanliness, poor processing, handling conditions and poor hygienic practices by the concerned employees. However, the microbes found in pasteurized milk were undesirable. Therefore, frequent inspection of pasteurized milk should be carried out to check whether they meet the minimum legal standards in respect of microbiological quality. The overall hygienic conditions during pasteurization should also be monitored and the post-pasteurized contaminated milk must be boiled before consuming.

Trace metal concentrations in milk

Milk has property of retention of metals due to formation of bioactive (lipophilic) complex (Leeuwen and Pinheiro, 2001; Buechler *et al.*, 2002). The average concentrations of trace elements in raw and pasteurized cow milk are shown in Table 3. Manganese is an essential nutrient for normal processes in the human body. But longtime exposure to high concentration of manganese can cause impaired neurological and neuromuscular control, mental and emotional disturbances. In the present study, Mn level in both raw (0.2 mg/L) and pasteurized milk (0.21 mg/L) was found to be higher than that of permissible limit of 0.05 mg/L (WHO, 1989) and pasteurization has no significant effect on manganese level in milk. Etonihu and Alicho (2010) found 1.17 mg/L Mn level in cow milk.

The copper is an important trace element acts as catalytic agent in the metabolism of hemoglobin, must be present in milk with a minimum concentration of 0.1 mg/L reported by Folley and Otterby (1978). Recorded data from the present study indicates that concentration of Cu in raw milk (0.17 mg/L) was significantly lower than pasteurized milk (0.24mg/L) and Cu concentration in both raw and pasteurized milk were within the safe limit of 1.00 mg/L set

by WHO (1989). Etonihu and Alicho (2010) found higher copper content (0.67 mg/L) in cow milk compare to present findings.

Iron is an important element in animals because of its presence in hemoglobin for respiratory and blood circulatory processes. Target organ are liver, kidney and the cardiovascular system for Fe toxicity. Concentration of Fe in both raw (10.02 mg/L) and pasteurized milk (10.37 mg/L) was not significantly different. Abd- El Aal *et al.*, (2012) found 5.30 and 5.68 mg/L Fe content in raw and sterilized cow milk respectively. The levels of iron in this study were higher than the recommended safe limit of 0.3 mg/L (WHO, 1989).

In pasteurized milk, zinc content (3.77 mg/L) was significantly higher than raw milk (2.72 mg/L) in the present study. It is reported that the mean Zn concentration in raw bovine milk is ranged from 0.29 to 4.96 mg/L (Licata *et al.*, 2004) and in cow milk is 0.20 mg/L (Etonihu and Alicho, 2010). The present Zn concentration is lower than permissible limit of 5.00 mg/L recommended by WHO (1989).

Calcium is an important regulator of normal cardiac rhythm maintenance, blood clotting, hormone secretion, muscle contraction and enzyme activation (Cashman and Flynn, 2002). Decreased concentration of calcium was found lower in raw milk (616.41 mg/L) than in pasteurized milk (756.51mg/L) and there was a significant difference in calcium content between raw and pasteurized milk. Calcium level in the present study was below the minimum recommended value (1300 mg/L) for calcium in milk, according to Folley and Otterby (1978). Soares *et al.*, (2010) found 888 mg/L Ca content in pasteurized bovine milk. It is reported that pasteurization reduces bioavailable calcium in the milk (Battestin *et al.*, 2002). On the other hand, Claeys *et al.* (2012) reported that pasteurization has no significant effect on the amount or bioavailability of calcium.

Magnesium is an essential element for oxidative phosphorylation and for various enzymatic reactions. In this study, magnesium content was 51.30 mg/L in pasteurized milk which was significantly different (<0.05) from that of raw milk (45.23 mg/L). Vahčić *et al.* (2010) found 110.8 mg/L magnesium in

Table 4. Daily intake of metal by consumption of milk and Health Risk Index (HRI) for heavy metals in raw and pasteurized milk

Sample	Daily Intake of metal (DIM) mg/person/day								
	Pb	Cd	Cr	Cu	Co	Ni	Fe	Zn	Mn
Raw milk	0.00169	0.000163	0.00052	7.4E-05	0.000445	0.00076	0.00437	0.00119	8.7E-05
Pasteurized milk	0.00147	0.000134	0.00054	0.0001	0.000646	0.00066	0.00453	0.00165	9.2E-05
HRI (mg/person/day)									
Raw milk	0.42292	0.163277	0.000350	0.001855	0.000147	0.0382	8.74889E-05	0.003958	0.00264
Pasteurized milk	0.36781	0.13490	0.000360	0.01615	0.000214	0.03296	9.05449E-05	5.48625E-05	0.002778

Values are the mean of three replicates; SD =Standard Deviation

pasteurized cow milk. The chromium is an important metal for the homeostasis of serum cholesterol and glucose tolerance. Its disability can cause retardation in the growth and commitment of protein and lipid metabolism (Picciano, 1985). But longterm exposure of chromium can cause kidney and liver damage as well as circulatory and nerve tissue problems. The average concentration of chromium was slightly lower in raw milk (1.20 mg/L) than pasteurized milk (1.24 mg/L). The difference however was not statistically significant. The level of chromium in both raw and pasteurized milk was above the acceptable limit of 0.05 set by WHO (1989). Etonihu and Alicho (2010) found chromium level of 0.15 mg/L in cow milk whereas Ogabiela *et al*. (2011) found high concentration of Cr (1.76 mg/L) in cow milk from Kano of Nigeria.

Small amount of nickel is needed to produce red blood cells in the body. Long term exposure of nickel causes skin irritation, heart and liver damage. The current result revealed that Ni content ranged from 1.51 (raw milk) to 1.75 (pasteurized milk) higher than the permissible limit of 0.02 mg/L set by WHO (1989). The difference of nickel content between raw and pasteurized milk was not statistically significant. Nickel result was nearly similar in cow milk found by Etonihu and Alicho (2010). The present result showed that cobalt content in pasteurized milk (1.48 mg/L) was significantly higher than raw milk (1.02 mg/L). Etonihu and Alicho (2010) found 0.06 mg/L cobalt content in cow milk. The present cobalt content was above the acceptable limit of 0.02 mg/L (WHO, 1989).

Besides essential elements, the toxic elements like lead, cadmium, arsenic and mercury were also investigated in the present study. These elements are not degradable absolutely and therefore they can be accumulated in the environmental components and can transport from one place to another place by air or as a gas or as absorbed in particular materials (Baird, 2002). Statistical analysis showed that lead concentration in raw milk (3.88 mg/L)

was significantly higher than in pasteurized milk (3.37 mg/L). Freezing or heating did not make any significant changes in lead concentration in cow's milk (Mata *et al.*, 1996). The desiccation process may produce significant increases in lead concentration (Moreno Rojas *et al*., 1999). The presence of Pb with high concentration in cow's milk also may be due to consumption of contaminated water or to use of metallic utensil where the milk is kept. Present results showed that the level of lead was above the acceptable limit of 0.02 (EU regulation 1881/2006; FAO/WHO, 2011). Lead toxicity can reduce cognitive development and intellectual performance in children.

Cadmium concentration was significantly higher in raw milk (0.37 mg/L) compared to pasteurized milk (0.31 mg/L). However, Cd content in both raw and pasteurized milk within the recommended limit set by FAO/WHO (1984). The average concentration of Cd in raw cow's milk samples was 0.416mg/L obtained by Abd- El Aal *et al.*, (2012). Arsenic is a widely distributed metalloid which is regarded as human carcinogen from extremely low levels of exposure. It is usually occurring in rock, soil, water, and air. The observed arsenic level in both raw and pasteurized milk were less than detection limit (< 0.003μg/L) of AAS. There was a report that the permitted arsenic level should be less than 0.01 mg/kg (International Dairy Federation, 1986). Rosas *et al.* (1999) reported that As concentration in cow milk ranged from 0.0009 to 0.028 mg/kg. Higher intake of mercury is associated with damage to the central nervous system (neurotoxicity) and the kidney. The concentrations for Hg were less than detection limit of (< 0.3μg/L) in both raw and pasteurized milk.

Potential health risk assessment

To assess the health risk of inhabitants of the study area, the Daily intake of metal (DIM) through milk consumption and the Health Risk Index (HRI) were estimated.

Daily intake of metal

The daily intake of the metals through milk depends on both the concentration and the amount of milk consumed. Values of DIM calculated for Cr, Co, Ni, Cu, Pb, Cd, Zn, Fe and Mn for adult (70 kg) are presented in Table 4. In pasteurized milk, the measured daily intake of metals ranged from 0.000091 to 0.0045 mg/person/day whereas in raw milk, the range was 7.42×10^{-5} to 0.0043 mg/person/day. The present data revealed that the values of daily intake of Cr, Co, Ni, Cu, Pb, Cd, Zn, Fe and Mn are within the recommended value mentioned by different authors and organization (Food and Nutrition Board, 1980; Santos *et al.*, 2004; Farid *et al.*, 2004; FAO/WHO, 2007; Dawd, 2010; Ogabiela *et al.*, 2011). Thus, the inhabitants in the study area are not likely to be exposed to sicknesses from milk intake.

Health risk index

The health risk associated with the metal intake through food can be evaluated from a very useful index, the Health Risk Index (HRI) (USEPA, 2002; Cui *et al.*, 2004 and Wang *et al.*, 2005). The marginal value of the index is 1. The value less than 1is considered as safe for human health to consume such food and above 1 has been recognized as not safe for human health, having potential health risk (USEPA, 2002). The estimated HRI values for the studied elements for both raw and pasteurized milk were below 1 (Table 4). This clearly indicates that the local inhabitants are not likely to be exposed to potential health risk due to lower accumulation of heavy metals from milk of the experimental area.

Hazard index (HI)

The Hazard Index (HI) was calculated for the toxic element Cr, Co, Cu, Mn, Fe, Zn, Ni, Pb and Cd in raw milk (0.633451701) and pasteurized (0.555325434) milk. According to USEPA (1989) hazard index, below 1 having no adverse health effect. Thus, the results of the hazard index suggested that both raw and pasteurized milk collected from Savar area of Dhaka city are safe for consumption.

Conclusion

The physico-chemical characteristics and nutritional parameters of raw and pasteurized milk indicate that milk from selected farm maintained good quality standard and thus offers excellent opportunities for the development of local dairy industry and to meet the public need for nutrition.

The microbiological status showed that pasteurized milk contained less number of bacterial counts than the raw milk. Regarding to trace elemental analysis, though concentrations of Mn, Fe, Cr, Ni, Co, and Cd were above the suggested value in milk sample, however the values of the daily intake of metal and heath risk index of these elements indicate no health risk as well as safe for consumption. Level of Cu, Zn, Ca, As and Hg in both raw and pasteurized were below the recommended permissible limit. Although the number of samples analyzed is still small, the data give a first picture of trace elemental concentration and physico-chemical levels in milk frequently consumed by the inhabitants of prescribed area of Bangladesh. It would be of great interest to further investigate the quality parameters in milk of other regions of Bangladesh as well as helpful for safe consumption.

Acknowledgments

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